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CERTIFICATE

This certificate is issued in support of an application for Patent registration in a country outside New Zealand pursuant to the Patents Act 1953 and the Regulations thereunder.

I hereby certify that annexed is a true copy of the Provisional Specification as filed on 8 April 2003 with an application for Letters Patent number 525198 made by Auckland UniServices Limited.

Dated 31 March 2004.

Neville Harris
Commissioner of Patents, Trade Marks and Designs

COMMISSIONER OF PATENTS, TRADEMARKS & DESIGNS
NEW ZEALAND

330 High Street, Lower Hutt and 17 Toop Street, Seaview
PO Box 30687, Lower Hutt, New Zealand or DX SX 11129, Wellington
Phone: +64 4 560 1600. Fax: +64 4 560 1691. www.iponz.govt.nz

Patents Form No. 4

Patents Act 1953

PROVISIONAL SPECIFICATION

DNA analysis system

We, Auckland UniServices Limited, of Level 10, 70 Symonds St., Auckland 1000,

do hereby declare this invention to be described in the following statement:

DNA analysis system

Field of the Invention

This invention relates to DNA analysis. More particularly, the invention relates to a DNA analysis system.

Background to the Invention

With the identification of the structure of DNA, research and development in the field of genetics at a molecular level was established.

To analyse DNA from a sample or organism traditionally requires many different steps. It also requires at least three different items of equipment excluding the equipment used to display the result. The use of three separate automated instruments to perform different parts of the analysis process renders the equipment bulky and unable to be used in the field. Also, because the instruments are so large, it would not achieve any useful purpose to integrate them into a single unit or system. In addition, the equipment requires substantial technical expertise to operate. Therefore, most of these instruments are built for use in laboratories. A sample that requires analysis must be collected at the site and sent to the laboratory. This can, in certain circumstances, be undesired such as, for example, at a crime scene where delays in obtaining information can lead to loss of valuable time in investigating the matter.

Still further, in the preparation of the sample for analysis purposes, a quantity of the sample is placed in a test tube which needs to be sealed and opened at intervals to add agents. Certain of these agents, apart from being toxic, need to be removed prior to analysis to inhibit contamination. Also, the need continuously to open and close the

test tube containing the sample renders the sample vulnerable to being contaminated which can adversely affect the final result.

Summary of the Invention

Broadly, according to a first aspect of the invention, there is provided a DNA analysis system which includes a unit that effects both extraction of DNA and identical replication of a region of interest of extracted DNA strands.

More specifically, according to a first aspect of the invention, there is provided a DNA analysis system which includes:

a thermal cycler operable as an extraction stage for extracting DNA from a sample to be tested and as an amplification stage for replicating identically a region of interest in DNA strands extracted from the sample;

a purification stage for purifying the amplified material from the thermal cycler; and

an analysis stage for analysing the purified sample to obtain genetic information relating to the sample.

The system may be used for detecting the presence of predetermined sequences such as pathogens. For this purpose, the amplification stage may include nucleotide sequence detection for the purpose of looking for specific sequences associated with those pathogens, etc. Nucleotide sequence detection may therefore be performed during the amplification stage, by adding fluorescently labelled oligonucleotides that can target any specific short sequence of DNA. The thermal cycler used in this case

may include an attached fluorimeter and light source. In this case, the later stages of the system may be omitted.

The analysis stage may comprise a separation stage and a detection stage.

The use of the thermal cycler both for the extraction stage and the amplification stage may be facilitated by the use of a non-specific thermophilic enzyme, the thermophilic enzyme being stable and active in a temperature range of about 65-80°C but which is denatured at a temperature exceeding about 90°C.

Broadly, according to a second aspect of the invention, there is provided a method of preparing a sample for DNA analysis, the method including the step of using a single unit to effect both extraction of DNA and identical replication of a region of interest of extracted DNA strands.

More specifically, according to a second aspect of the invention, there is provided a method of preparing a sample for DNA analysis, the method including the steps of:

placing a sample of material to be analysed in a thermal cycler and adding a predetermined quantity of a non-specific thermophilic enzyme, as described above, to the thermal cycler;

cycling the mixture through a predetermined temperature profile to effect extraction of DNA material from the sample;

in the thermal cycler, subjecting the extracted DNA material to an amplification stage replicating identically a region of interest in the extracted DNA material; and

sequencing the amplified material.

The material may be sequenced by a dideoxy method of sequencing which includes the steps of sequencing, separation and detection.

Alternatively, the amplification stage may include nucleotide sequence detection for the purpose of looking for specific sequences such as those associated with predetermined pathogens, etc. The method may include performing nucleotide sequence detection during the amplification stage, by adding fluorescently labelled oligonucleotides that can target any specific short sequence of DNA. The method may include using a thermal cycler that has an attached fluorimeter and light source. In this case the later steps of the method may be omitted.

The method may include, prior to separating the DNA material, purifying the material and sequencing the purified DNA material.

The step of sequencing the purified DNA material for separation and detection may be effected in the thermal cycler.

In this specification, the non-specific thermophilic enzyme is referred to as "proteinase". More particularly, the proteinase used in the system is described in greater detail in International Patent Application No. PCT/NZ02/00093 to The University of Waikato. The contents of that patent application are incorporated herein by reference.

As indicated above, preferably, the thermal cycler is also used for sequencing prior to the separation stage. Thus, one piece of equipment, being the thermal cycler, may be used for extraction, amplification and sequencing. Also, due to the fact that the proteinase is denatured during the extraction phase, the need for a centrifuge to separate out impurities from the sample is obviated.

The thermal cycler may include a control means in the form of a microcontroller which controls the various stages of preparation of the sample.

Further, the thermal cycler may include a heating mechanism for heating the sample, contained in one or more vials or test tubes, received in the thermal cycler. The heating mechanism may be controlled by the microcontroller to maintain the sample at the required temperatures at the various stages of extraction, amplification and sequencing.

The system may include a monitoring means for monitoring the analysis stage. The monitoring means may be in the form of a computer having a display on which data relating to the analysed sample are displayed.

The purification stage may incorporate a size filtration matrix comprising a gel filtration media incorporating a filtering resin. This matrix may allow larger fragments of DNA through from the amplification stage before any smaller fragments and other unwanted substances. The larger fragments may be collected for use in the sequencing stage. The sequencing stage may encompass tagging ends of the fragments with dideoxynucleoside triphosphates (ddNTP's) labelled with different fluorochromes before being graded.

The grading may form the first step of the separation stage and may incorporate separating the fragments into fragments of differing lengths by a separation device. The separation device may be an electrophoresis device. The electrophoresis device may be a capillary electrophoresis device and may include a detecting means for detecting information relating to tagged fluorescent nucleotides at the end of each of the DNA fragments.

The detecting means may include a laser device that irradiates the ends of the DNA fragments to cause the fluorescent ends to fluoresce. The fluorescent ends may be read by a detector in the form of a charge coupled device (CCD) camera or a photomultiplier tube (PMT), the output of which is fed to the monitoring means.

Brief Description of the Drawings

The invention is now described by way of example with reference to the accompanying diagrammatic drawings in which:-

Figure 1 shows a schematic representation of a DNA analysis system, in accordance with the invention; and

Figure 2 shows a time-based schematic depiction of the operation of the system.

Detailed Description of the Drawings

In the drawings, a DNA analysis system, in accordance with the invention is illustrated and is designated generally by the reference numeral 10. The system 10 includes a thermal cycler 12 and a monitoring means in the form of a computer 14. As illustrated more clearly in Figure 2 of the drawings and as will be described in greater detail below, the thermal cycler 12 is used, initially, for an extraction stage 16 followed by an amplification stage 18 followed by a sequencing stage 20.

A purification stage 22 is interposed between the amplification stage 18 and the sequencing stage 20. It is emphasised that, what is illustrated in Figure 2 of the drawings, is a time-based illustration of the sequence of events leading to analysis of

DNA material. The thermal cycler 12 is used for all three of the extraction stage 16, the amplification stage 18, and the sequencing stage 20.

The thermal cycler 12 has a housing 24 on which a keypad 26 for controlling operation of the thermal cycler 12 is mounted. A receptacle 28 containing a plurality of test tubes (or wells) 30, in which sample material is received, is mounted on top of the housing 24. The receptacle 28 is closed by a heat control lid 32.

A remote controlled pipette 34 is mounted on an arm 36. The pipette 34 is used to inject sample material into the test tubes 30. The arm 36 is suspended from a beam 38. The arm 36 is displaceable horizontally along the beam 38 as indicated by arrow 40 under control of the computer 14 as illustrated by control line 42. In addition, the pipette 34 can also move vertically on the arm 36 as indicated by arrow 44, once again, under control of the computer 14.

As illustrated in Figure 2 of the drawings, the thermal cycler 12 includes a plurality of heating elements 46 and a thermocouple 48.

In use, a sample 60 of material to be analysed is inserted into one or more of the test tubes 30 of the thermal cycler 12. The sample could be a bacterial or cultural swab 52, human or animal tissue 54, which has been homogenised as shown at 56, or human or animal blood 58. For ease of explanation, the sample will be referred to by reference numeral 60.

The sample 60 is inserted into the thermal cycler 12 together with an extraction solution 62.

The extraction solution 62 comprises proteinase as defined above. One µl of proteinase is added together with each unit of sample material 60. The extraction solution 62 further comprises 100 µl of buffer for each µl of proteinase.

The solution in the test tubes 30 of the thermal cycler 12 is then subjected to 15 minutes of heating at about 75°C. At this temperature, the cells of the sample material 60 are lysed to facilitate extraction of DNA material. Once the DNA material has been extracted from the cells of the sample material 60, the proteinase is denatured by subjecting the solution to heat at about 95°C for a further 15 minute period.

Approximately 1-5 µl of extracted material in solution 64 is then subjected to the amplification stage 18. The amplification stage 18 is a polymerase chain reaction (PCR) amplification stage for effecting rapid replication of a specific region of the DNA material. The solution 64 may be diluted, if necessary, so that only a small quantity of DNA contained in the solution 64 is carried forward to the following stage.

In the amplification stage 18, the solution 64 is mixed with a master solution 66. Approximately 20 µl of master solution 66 is used together with the 1-5 µl of solution 64. The master solution 66 comprises a buffer, an enzyme - Taq DNA polymerase, two oligonucleotide primers, deoxynucleoside triphosphate (dNTPs) and a cofactor $MgCl_2$.. The primers determine which region of the DNA material is to be amplified.

Finally, the master solution includes Taq DNA polymerase.

In the amplification stage 18, the solution, being a combination of the solutions 64 and 66, is heated firstly to a temperature in a range of about 94-96°C, preferably 94°C, for 30 seconds to denature the target DNA. The temperature is lowered to a temperature in a range of about 50-65°C, preferably about 55°C, for a further 30

seconds to permit the primers to anneal to their complementary sequences. Finally, the temperature is raised to a temperature of about 72°C for a further 30 seconds to allow the Taq DNA polymerase to attach at each primed site and to form a new DNA strand. The cycling through the various temperatures is repeated approximately 30 times so that the DNA material is multiplied more than a billion times.

The amplified solution 68 is fed from the amplification stage 18 to the purification stage 22. Once again, approximately 1-5 µl of solution 68 is fed through the purification stage 22. The purification stage 22 comprises a gel filtration device 70. The filtration device 70 is in the form of a tube 72 containing a quantity of gel filtration media 74. A valve 76 controls the passage of the solution 68 through the tube 72. A waste valve 78 is provided through which waste material can be discharged to a container 80 to remove the dNTPs, primers and reaction products other than the material of interest.

In the purification stage, the gel filtration media 74 allows the larger fragments of DNA through before allowing any smaller fragments, dNTPs and primers through.

A suitable gel filtration medium is a resin composed of macroscopic beads synthetically derived from the polysaccharide, dextran such as that sold under the trade name, Sephadex G50/G25 (Sephadex is a registered trade mark of Amersham Biosciences AB, Uppsala, Sweden).

The larger fragments of DNA are collected at the downstream end of the tube 72 for sequencing in the sequencing stage 20.

In the sequencing stage 20, the DNA in the solution 82 is sequenced into many pieces of differing lengths using restriction enzymes. Each piece is used as a template

to generate a set of DNA fragments where any one DNA fragment differs in length from any other DNA fragment by a single nucleotide base.

The nucleotide base at the end of each of the DNA fragments is tagged with one of four dideoxynucleoside bases (ddATP, ddTTP, ddCTP, ddGTP). Since each of the four nucleoside bases contains a different dye, when excited with a laser, the bases emit light at different wavelengths. For this purpose, the system 10 has a supply 84 of a solution containing dyes which is fed into the thermal cycler to effect sequencing. A suitable sequencing solution that can be used is Big-Dye (Big-Dye is a trade mark of Applied Biosystems, USA). The sequencing solution is mixed in a quantity of about 20 µl with the solution 82 to dye the nucleotide bases at the ends of the DNA fragments.

To randomly terminate the nucleotide bases and fluorescently label the ends of the DNA fragments, the solution in the thermal cycler 12 is cycled through a temperature of approximately 96°C for about 30 seconds followed by a temperature of approximately 50°C for about 15 seconds followed by a temperature of approximately 60°C for about 4 minutes. This cycle is repeated approximately 25 times.

The solution 86 with the fluorescently labelled DNA fragments is fed from the thermal cycler 12 into a separation stage of an analysis stage 88 of the system 10. The separation stage 88 makes use of electrophoresis equipment, more particularly, capillary electrophoresis equipment. The equipment 90 includes a capillary 92, containing polyacrylamid or agarose gel, having an upstream end in a sample vial 94 into which the fluorescently labelled DNA fragments are fed from the sequencing stage 20. The DNA fragments are fed through the capillary 92 into an output vial 96. As the solution 86 moves through the capillary 92, the solution 86 is subjected to a high

voltage field provided by a high voltage power supply 98. The power supply 98 provides a voltage in the region of 5-30 kV. As the DNA fragments migrate through the capillary 92 and, because the DNA fragments are of different lengths, they take different amounts of time to migrate from one end of the capillary 92 to the other end.

The analysis stage of the system 10 includes a detecting stage, or detector, 110 for detecting and reading the nucleotide bases of the DNA fragments. The detector 110 comprises an excitation source in the form of a laser 100 to excite the fluorescently labelled ends of the DNA fragments. Thus, the DNA fragments passing through the capillary 92 are subjected to laser light from the laser 100. The detector 110 further includes a reading means in the form of a CCD camera 102, and/or a spectrograph or one or more photomultiplier tubes (PMTs) for reading the wavelength of the fluorescing material. An output 104 from the camera 102 is fed to the computer 14 where an electropherogram, 106 is displayed on a screen 108 of the computer 14 representative of the DNA sequence of the sample 60. Software of the computer converts the collected data into sequence information using a base-calling algorithm to produce the electropherogram. The electropherogram is a plot of sequence data.

It will be appreciated that the electropherogram 106 is generated by reading off the light from a final nucleotide base at the end of each DNA fragment. Since each base is tagged with a different colour, it is possible to detect the order of the nucleotide bases in the DNA fragment sequence.

Because of the use of the proteinase, as defined above, a system 10 is provided which makes use of the thermal cycler 12 for effecting extraction, amplification, and sequencing using a single device. Hence, a portable, field-useable, system 10 is provided which requires minimum human intervention. More particularly, the need to

open the test tubes or wells 30 containing the samples material 60 regularly is obviated thereby reducing the risk of contaminating the sample material 60 to be analysed.

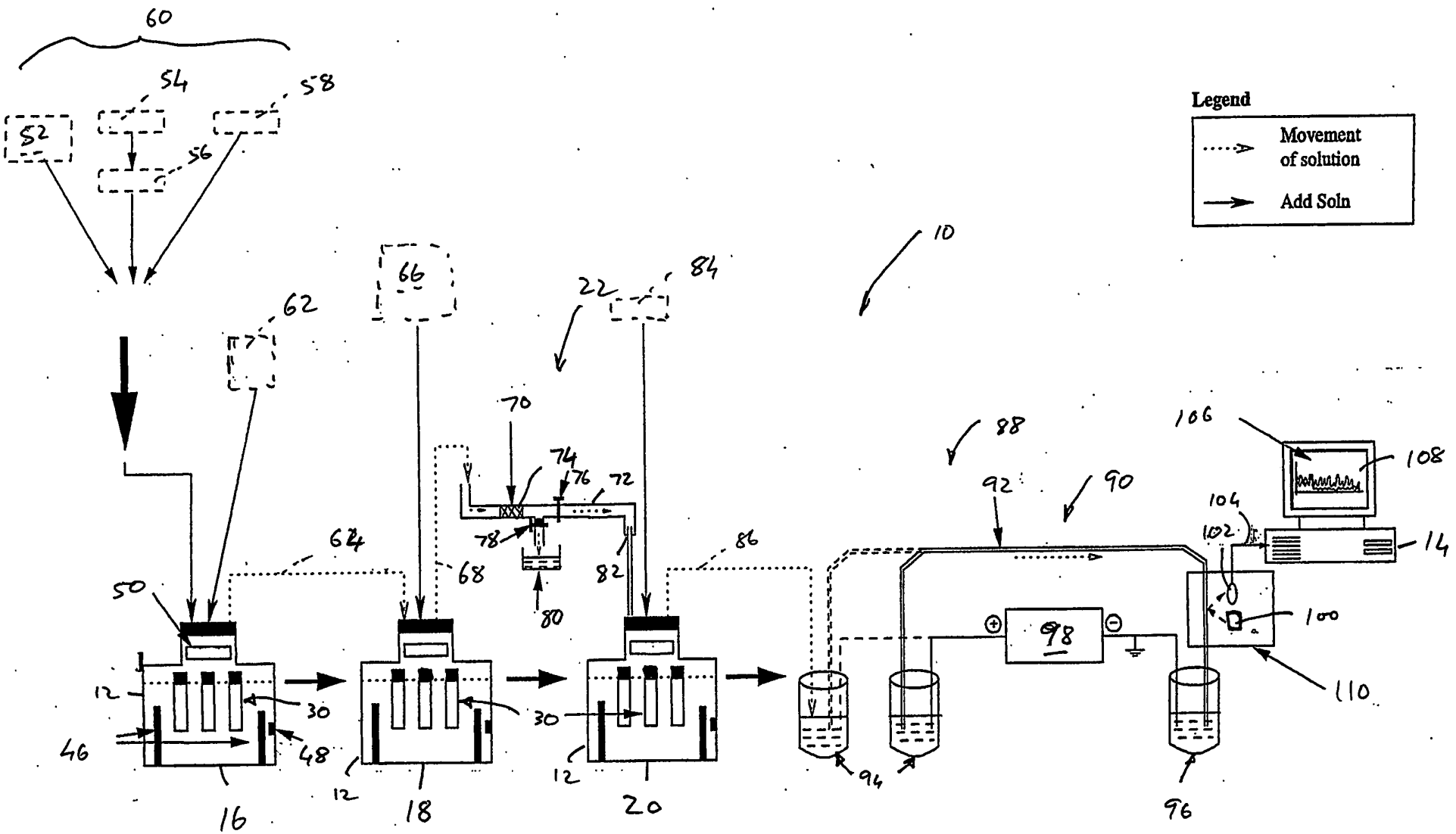
Still further, because the proteinase is denatured in the extraction phase, it is not necessary to make use of separating equipment such as centrifuges. This further reduces the size and weight of the system 10 rendering it portable.

Hence, it is a particular advantage of the invention that a portable DNA analysis system is provided. The system is integrated and requires very little human intervention or expertise to operate. The benefit of an integrated system is a reduction in the number of components and also the costs of conducting the analysis by reducing the labour costs and sample reagent consumption.

Such a system is particularly useful in fields such as health care, agriculture, forensic medicine, military applications, environmental monitoring, animal husbandry, or the like. The use of a portable system provides the ability for analysis to be done in situ with the resultant, self-evident advantages.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

FIG. 1


Legend
Movement of solution
Add Soln
60
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94
98
100
110
96

FIG. 2